# Pathological Impacts Due to The Existence of Plastic Waste in Rumen of Bali Cattle

I Ketut Berata<sup>1</sup>\*, Ni Nyoman Werdi Susari<sup>1</sup>, I Made Kardena<sup>1</sup>, Arindita Niatazya Novianti<sup>2</sup>

<sup>1</sup>Faculty of Veterinary Medicine, Udayana University, Jimbaran, Badung, Bali, Indonesia, <sup>2</sup>Division of Veterinary Basic Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. \*Corresponding author: berata\_iketut@unud.ac.id

#### Abstract

This study aimed to determine the prevalence of Bali cattle rumen containing plastic waste and its impact on tissues and health risks. A total of 100 Bali cattle were investigated after being slaughtered at several traditional slaughterhouses in Denpasar City. Rumen samples were examined for any plastic waste, meanwhile blood, liver, kidney, lungs, spleen, intestine, and myocardium were collected for evaluation of heavy metals content and histopathological examination. The heavy metal measurement was performed using the atomic absorption spectrophotometry (AAS) method. Those tissues for histopathological examination were fixed in buffer neutral formalin (BNF) and then evaluated using hematoxylin-eosin (HE) staining. The results of the examination reported 9 rumens (9%) containing plastic waste. The hematological profile showed leucocytosis and the content of lead (0.841  $\pm$  0.522 ppm). Histopathological changes in the liver, kidneys, lungs, spleen, and intestine, were found in various types of degeneration, inflammation, and mild necrosis. In conclusion, the prevalence of plastic waste in the rumen of Bali cattle was 9%, with leucocytosis, lead content was 0.841  $\pm$  0.522 ppm, and various histopathological lesions such as congestion, inflammation, and fatty degeneration in parenchymous tissues.

Keywords: heavy metals, histopathology, plastic waste, rumen, health risk

Received: 10 June 2023

Revised: 26 October 2023

Accepted: 27 November 2023

#### INTRODUCTION

The presence of plastic waste in the digestion of most rumen is indicated by a poor cattle environment. The prevalence of cattle rumen containing plastic waste in Nairobi Kenya receives more than 30% (Lange *et al.*, 2018). Rumen inspection results on cattle slaughtered at slaughterhouses in Bali still found plastic waste and other inorganic materials. Place the plastic waste within the beef rumen, which is robust due to the plastic-wrapped fodder (Wiradana *et al.*, 2023).

The health status of cattle is very much determined by the factors of food sources and the environment. The results of studies of cattle kept at the final disposal site of Denpasar City found the presence of lead contamination in their blood (Berata *et al.*, 2016) and some of them in the rumen found plastic waste. The urban waste consists mainly of inorganic material that is difficult to degrade, so it becomes a threat for the

animals foraging (Dinana *et al.*, 2023). Some of the inorganic materials that are widely studied in cattle that are reared in landfills are the presence of heavy metals lead and cadmium. Both of these heavy metals are very dangerous to human health when consuming polluted beef (Sharma *et al.*, 2014). The difficulty in tracing the origins of cattle slaughtered at the slaughterhouse, so the presence of plastic waste in the rumen indicates that the rearing cattle are in an environment with a lot of inorganic material.

The impact of the presence of plastic waste in the rumen of cattle reportedly can disrupt the physiological and histopathological body tissues (Roggeman *et al.*, 2013). Metabolic irregularities due to intake of contaminated feed including plastic waste, will cause animals more sensitive to infectious diseases. Hepatotoxic due to toxicity originating from inorganic substances, can cause a decrease in cattle immunity (Loh *et al.*, 2020). This study investigated the prevalence of rumen containing plastic waste, hematologic profile, and histopathological features of liver, kidney, spleen, and lung tissue.

#### MATERIALS AND METHODS

# **Ethical Approval**

All procedures for using experimental animals have received approval from the Veterinary Ethics Committee, Faculty of Veterinary Medicine, Udayana University, with Approval No. B/37/UN14.2.9/PT.01.04/2022.

# **Study Period and Location**

The study was conducted in December 2022. The study used a sample of 100 Bali cattle, which were slaughtered at a traditional slaughterhouse in the Banjar Bersih, Darmasaba Village, Badung Regency. From each of these cattle were collected the blood, liver, kidney, spleen, lung, and muscle tissues.

# **Sample Evaluation**

The examination of the hematological profile was carried out at the Denpasar Veterinary Center, using the Auto Analyzer Refloton (R) method. The variables examined in the hematological profile were total erythrocytes, hemoglobin, mean corpuscular volume (MCV), lymphocyte percentage, monocytes, and basophils.

To measure of lead heavy metals content was carried out at the Analytical Laboratory of Udayana University. The method used was an atomic absorption spectrophotometer (AAS) (Sikiric *et al.*, 2013).

For histopathological evaluation, fresh tissue was collected as follows liver, kidneys, spleen, lungs, and muscles, then fixed in 10% formalin neutral buffer (NBF) for 24 hours. Furthermore, it was evaluated using hematoxylin-eosin (HE) staining (Hristu *et al.*, 2021).

# Data Analysis

Data on hematologic profiles, measurements of heavy metal levels, and histopathological analysis were tabulated and analyzed descriptively and qualitatively.

# **RESULTS AND DISCUSSION**

Nine (9%) of the 100 cattle whose rumens were examined had plastic waste in them. Ropes and shattered plastic bags are two examples of plastic waste. The results of examining the blood profile of cattle whose rumen contains plastic waste are presented in Table 1.

The results of the measurement of lead levels indicate variations in the level of contamination. None cadmium contamination was found in all tissues. Complete levels of lead in each network can be seen in Table 2. Histopathological changes observed obtained results that vary greatly from mild to moderate. A description of the histopathological changes in each tissues are presented in Table 3.

The prevalence of plastic waste in the rumen of as much as 9% shows that there are still cattle farms in the environment where there is plastic waste. This prevalence data is much lower than that reported in Kenya at 30.42% (Lange *et al.*, 2018). With the issuance of Bali Governor Regulation No. 97 of 2018 concerning restrictions on disposable plastic waste heaps, it is expected that the prevalence of plastic waste in cattle rumen in Bali can decrease.

Based on hematological profile data, it was found that the percentage of leukocytes especially lymphocytes and monocytes was higher than the normal standard. This shows that there is an inflammatory response in the body of a cattle in its rumen containing plastic waste. Particles of plastic components can cause various negative impacts on the body's physiological system, even to the point of immunosuppression (Priyanka and Dey, 2018).

The inflammatory response can be caused by damage to cells that can cause tissue necrosis. The lead tends to be higher in the blood than in tissues, because lead is substituted for Fe in hemoglobin, and incidentally gets bound in the tissue (Coates, 2014). Variations in lead levels between tissues indicate differences in endurance or connective tissue with lead. There is a character of each tissue in the binding lead (Pilarczyk *et al.*, 2013). The level of lead in the tissue is different from the results reported (Akan *et al.*, 2010), which states

	Parameters						
No	Ε	Hb	MCV	L	Lf	Mn	Bf
	$(10^{6}/mm^{3})$	(g/dL)	(mL x 10 <sup>12</sup> )	$(10^{3}/uL)$	(%)	(%)	(%)
1	7.84	12.6	54.1	11.91	86.8	6.5	6.8
2	5.56	10.6	56.3	7.77	80.9	7.1	5.5
3	6.02	11.5	55.3	7.10	81.5	6.9	4.9
4	4.95	10.0	57.0	7.69	83.9	10.3	5.6
5	5.25	10.5	56.0	9.66	80.5	9.1	6.1
6	4.98	10.3	54.5	6.50	84.4	4.5	4.5
7	6.85	14.0	56.5	6.85	88.3	7.4	2.4
8	6.75	14.0	51.4	6.75	81.2	4.7	11.4
9	6.81	13.9	54.9	6.81	82.5	5.0	4.9
Mean	6.11	12.0	55.11	8.40	83.3	6.8	5.8
Normal	5–8	9–14	50-60	8.00	58.0	4.0	0–5

<b>Table 1.</b> Hematology profile of cattle whose rumen contains plastic waste
---

(E) erythrocytes, (Hb) hemoglobin, (MCV) mean corpuscular volume, (L) leukocytes,

(Lf) lymphocytes, (Mn) monocytes, (Bf) basophils.

Table 2. Levels of lead heavy metals in the tissues of cattle

No	Lead level in the blood and tissues of cattle (ppm)							
	Blood	Liver	Kidney	Spleen	Lungs	Intestine	Myocard	
1	1.922	0.991	0.782	0.998	0.776	0.589	0.012	
2	0.488	0.322	0.131	0.346	0.334	0.248	0.000	
3	2.843	0.688	0.448	0.898	0.555	0.182	0.002	
4	2.764	0.656	0.618	0.788	0.654	0.212	0.041	
5	2.228	0.743	0.622	0.994	0.777	0.228	0.032	
6	1.388	0.876	0.569	0.980	0.687	0.437	0.012	
7	0.046	0.023	0.014	0.086	0.077	0.042	0.000	
8	0.884	0.644	0.486	0.884	0.682	0.224	0.004	
9	2.089	1.192	0.984	0.999	0.741	0.324	0.008	
Mean	1.628	0.682	0.517	0.775	0.587	0.276	0.012	
	$\pm 0.990$	±0.347	±0.299	±0.331	±0.236	$\pm 0.158$	±0.015	

# Table 3. Histopathological change of the tissues of cattle

No	Congestion	Fat degeneration	Inflammation	Necrosis
1	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	n/a
2	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	Intestine
3	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	Intestine, lungs
4	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	Intestine
5	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	Intestine
6	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	Liver, intestine
7	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	Intestine
8	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	Liver, kidney, intestine
9	Liver, kidney, lungs	Liver, kidney	Liver, kidney, lungs, intestine	n/a

the liver and kidney contain the highest lead. Meanwhile, the results showed the highest in the spleen and lungs (Kristanto and Septiyani, 2023).

The spleen is the body's defense organ as well as a hemopoietic organ. If it is associated with the mechanism of lead in the substitution of Fe in hemoglobin, then the hematopoietic tissue is most damaged by the contamination of lead. This is consistent with what was reported that heavy metals will accumulate in tissues that act as hemopoietic (Li et al., 2015). Other study reported that lead accelerates the absorption of spleen erythrocytes so that lead accumulates in the spleen (Purnama et al., 2019). There are similarities with the results of this study that lead levels in muscle are the lowest (Jaishankar et al., 2014). In fish, it was reported that the lowest tissue velocity as a place of lead accumulation was in the muscles (Pebriani et al., 2022).

# CONCLUSION

The prevalence of cattle rumen containing plastic waste was 9%. The hematological profile of cattle which in their rumen contains plastic waste showed an increase in leukocytes. The lead content in the body tissues of cattle whose rumen contains plastic waste sequentially from the highest are blood, spleen, liver, lungs, kidneys, intestines, and myocard. Histopathological changes in the body tissue of cattle whose rumen contains plastic waste were dominated by congestion, inflammation fatty degeneration and mild necrose.

#### ACKNOWLEDGEMENTS

The authors thank The Rector of Udayana University through the Head of The Research Institute and Community Service of Udayana University, for funding this study.

#### **AUTHORS' CONTRIBUTIONS**

IKB: Conceptualization and drafted the manuscript. NNWS and IMK: Evaluated the samples. IKB and NNWS: Validation, supervision, and formal analysis. ANN: Performed the statistical analysis and the preparation of tables. All authors have read, reviewed, and approved the final manuscript.

#### **COMPETING INTERESTS**

The authors declare that they have no competing interests.

#### REFERENCES

- Akan, J. C., Abdulrahman, F. L., Sodipo, O. A., & Chiroma, Y. A. (2010). Distribution of heavy metals in the liver, kidney and meat of beef, mutton, caprine and chicken from Kasuwan Shanu Market in Maiduguri Metropolis, Borno State, Nigeria. *Research Journal of Applied Sciences, Engineering and Technology*, 2(8), 743–748.
- Berata, I. K., Kardena, I. M., Susari, N. N. W., & Sudira, I. W. (2017). Lead detection in blood of the Bali cattle that were slaughtered in the traditional slaughterhouses in Denpasar, Bali. *Bali Medical Journal*, 6(3), S43–S46.
- Coates, T. D. (2014). Physiology and pathophysiology of iron in hemoglobinassociated diseases. *Free Radical Biology and Medicine*, 72, 23–40.
- Dinana, Z., Rantam, F. A., Mustofa, I., & Rahmahani, J. (2023). Detection of Foot and Mouth Disease Virus in Cattle in Lamongan and Surabaya, Indonesia Using RT-PCR Method. *Jurnal Medik Veteriner*, 6(2), 191– 196.
- Hristu, R., Stanciu, S. G., Dumitru, A., Paun, B., Floroiu, I., Costache, M., & Stanciu, G. A. (2021). Influence of hematoxylin and eosin staining on the quantitative analysis of second harmonic generation imaging of fixed tissue sections. *Biomedical Optics Express*, 12(9), 5829–5843.
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014).

Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2), 60–72.

- Kristanto, D., & Septiyani. (2023). Comparison of Hematological Levels of Simmental-Ongole Crossbreed (SimPO) and Ongole Crossbreed (PO) Cattle Reared Semi-Intensively. *Jurnal Medik Veteriner*, 6(2), 237–243.
- Lange, N. C., Inganga, F., Busienei, W., Nguru, P., Kiema J., & Wahungu W. (2018). The prevalence of plastic bag waste in the rumen of slaughtered livestock at three abattoirs in Nairobi Metropolis, Kenya and implications on livestock health. *Livestock Research for Rural Development*, 30(11), 182.
- Li, Q., Liu, H., Alattar, M., Jiang, S., Han, J., Ma, Y., & Jiang, C. (2015). The preferential accumulation of heavy metals in different tissues following frequent respiratory exposure to PM2.5 in rats. *Scientific Reports*, 5, 16936.
- Loh, Z. H., Ouwerkerk, D., Klieve, A. V., Hungerford, N. L., & Fletcher, M. T. (2020). Toxin Degradation by Rumen Microorganisms: A Review. *Toxins*, 12(10), 664.
- Pebriani, M. A., Barus, T. A., & Ilyas, S. (2022). Fish diversity and heavy metal accumulation of Pb, Cu and Zn after Mount Sinabung Eruption in Benuken River, North Sumatra, Indonesia. *Biodiversitas*, 23(1), 187–194.
- Pilarczyk, R., Wójcik, J., Czerniak, P., Sablik, P., Pilarczyk, B., & Tomza-Marciniak, A. (2013). Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic

farm. *Environmental Monitoring and Assessment*, 185(10), 8383–8392.

- Priyanka, M., & Dey, S. (2018). Ruminal impaction due to plastic materials - An increasing threat to ruminants and its impact on human health in developing countries. *Veterinary World*, 11(9), 1307–1315.
- Purnama, M. T. E., Dewi, W. K., Prayoga, S. F., Triana, N. M., Aji, B. S. P., Fikri, F., & Hamid, I. S. (2019). Preslaughter stress in banyuwangi cattle during transport. *Indian Veterinary Journal*, 96(12), 50–52.
- Roggeman, S., van den Brink, N., Van Praet, N., Blust, R., & Bervoets, L. (2013). Metal exposure and accumulation patterns in freerange cows (*Bos taurus*) in a contaminated natural area: Influence of spatial and social behavior. *Environmental Pollution*, 172, 186–199.
- Sharma, B., Singh, S., & Siddiqi, N. J. (2014). Biomedical implications of heavy metals induced imbalances in redox systems. *BioMed Research International*, 2014, 640754.
- Sikiric, M. D., Brajenovicm, N., Pavlovic, I., Havranek, J. J., & Plavljanic, N. (2003). Determination of metals in cow's milk by flame atomic absorption spectrophotometry. *Czech Journal of Animal* Science, 48(11), 481–486.
- Wiradana, P. A., Sudyadnyana Sandhika, I.,
  Widhiantara, I. G., Rizqy, A. N., Soegianto,
  A., & Yulianto, B. (2023). Contaminants and
  Human Health Risks Associated with
  Exposure to Microplastic Ingestion of Green
  Mussels (*Perna viridis*) Collected from The
  Kedonganan Fish Market, Bali. Jurnal
  Medik Veteriner, 6(2), 197–208.

\*\*\*